

## REMARKS/ARGUMENTS

Claims 1-4, 6, 7, 16, 18, 20 and 21 are pending. Claims 1, 16, 18 and 20 have been amended. The specification has been amended. No new matter has been introduced. Reexamination and reconsideration of the present application is respectfully requested.

### 35 U.S.C. §112

The Examiner rejected to the claims under 35 U.S.C. 112. The Examiner states that the growth or culture is not claimed explicitly and thus there is no evidence that these limitations must be imported into the claims to give meaning to the disputed terms. Namely, the Examiner states that there must be a culturing or growing step at some point in reaching the invention. Thus, the Applicant has amended the claims to clarify that the identified strain must be obtained in a sufficient amount, either from isolation or culturing pursuant to the recited conditions, and that amount of bacterial strain is introduced into the aquarium. The disclosure shows supporting data as to the isolation steps as well as the culturing techniques that may be used. See Examples (specifically pp. 21-30).

The Applicant respectfully submits that while there is a culturing or growing step to achieve the initial bacteria biomass to introduce into the aquarium, there is no bacterial growth necessary for the nitrification process to occur. The Applicant submits additional experimental data to clarify this point, as well as to clarify the procedure and conditions in which the testing illustrated in the previously submitted chart (“Ammonia chloride/Nitrite kinetics in a breeding tank”) was conducted in. The performance of the test bacteria was evaluated by a “Flask-Test” method performed in laboratory and is described in the included protocol. The test evaluates how the concentration of the substrate  $\text{NH}_2\text{Cl}$  is transformed into nitrite and later from nitrite into nitrate. The three charts illustrate the results and demonstrate that nitrification is immediate upon introduction into the aquarium of the claimed bacterial strain and the entire nitrification process is completed in less than 1 day. As nitrifiers need about 12-14 hours per propagation cycle, it is clear that the nitrification could not be a result of intense population growth and formation of more biomass. The experimental data supports that the bacterial concentration does

not increase in correlation with the nitrification that occurs. Thus, the Applicant respectfully submits that once the sufficient amount is introduced into the aquarium, that amount of bacterial strain starts the nitrification upon introduction and there is no further growing or culturing necessary within the aquarium. The Examiner had indicated, in a telephonic interview on June 6, 2007, her concern that just one or two bacterial cells would not be enough to perform the invention. The Applicant addresses this by having the claim language expressly recite that there be a sufficient amount to alleviate or prevent the accumulation of ammonia in the aquarium which is taught by the disclosure and specifically, the various Examples.

In light of the above considerations, the Applicants respectfully submit that the claims, as amended, comply with the requirements of 35 U.S.C. 112.

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Conclusion

This response is being submitted within the three month deadline. In the case any fee is owed, please charge deposit account number 03-3975 (ref. 81289-294309). The Applicant believes that the claims are now in condition for allowance, and a favorable action is respectfully requested. If, for any reason, the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (213) 488-7100 to discuss the steps necessary for placing the application in condition for allowance should the Examiner believe that such a telephone conference would advance prosecution of the application.

Respectfully submitted,

PILLSBURY WINTHROP SHAW PITTMAN LLP

Date: 6/25/2007

By:   
Carolyn S. Lu  
Registration No. 56,817  
Attorney For Applicant(s)

725 South Figueroa Street, Suite 2800  
Los Angeles, CA 90017-5406  
Telephone: (213) 488-7100  
Facsimile: (213) 629-1033

Attachments: Laboratory protocol for testing nitrification (Flask-Test)

Chart illustrating test results (3)



## Ammonia and Nitrite Oxidation Flask Test for BioSpira QC for CRD

### Materials needed:

- 50 mg/ml ammonia-N Ammonium Chloride Stock: (19.24g ammonium chloride in one liter deionized water)
- (Sodium bicarbonate)
- 500 ml Erlenmeyer flasks:
  - 2 replicates of each treatment
  - Treatments are:
    - BioSpira (test bacteria), application for 120L aquarium tank tested at 140ml per 1L
    - Negative Control (no bacteria)
- Stir plate (preferably a multi-position stir plate so all replicates are treated the same)
- stir bars
- 10 ml Pipetman
- 0.20µm syringe filters
- 10ml syringe
- Sample tubes
- Graduated cylinders
- Containers to hold water
- BQS Tank Water
- Method and equipment to measure ammonia-N, nitrite-N, and nitrate-N
- pH Meter

### Method:

1. Thoroughly clean 500 ml Erlenmeyer flasks and sterilize it 2 hours at 200 °C.
2. Number flasks. Randomly assign each flask number a treatment.
3. Make a 3 L solution of 5 mg/L ammonia-N by diluting 3 ml 50 mg/ml ammonia-N stock solution to 3 L with BQS Tank Water (in a beaker).
4. Measure the pH value. (Add sodium bicarbonate until pH is ~8. Make sure solution is mixed well.)
5. Take two baseline samples from the beaker, measure with IC
6. Using a graduated cylinder, aliquot 200 ml of the 5 mg/L ammonia-N solution to each flask.
7. Add 1.0 ml of well mixed BioSpira to appropriate flask.
8. Add stir bars to each flask and place flasks on stir plate. Spin at medium-low speed for mixing and oxygenation. Cover the top of the flasks with a cap.
9. **Sample each flask in 24 hour intervals** for 10 days.
  - a. Using 10ml Pipetman, remove 10 ml from each flask and put it in a 30 ml plastic beaker.
  - b. For **Anion IC** take 4 ml with a syringe and filter it through a 5 µm filter, IC-H<sup>+</sup> cartridge and a 0.2 µm filter into a sample tube.  
Syringe, 5 µm filter, IC-H<sup>+</sup> cartridge and a 0.2 µm filter will be cleaned with deionized water after each sample.
  - c. For **Kation IC** add 2 drops of 1 M HNO<sub>3</sub> into the plastic pitcher, mix it carefully, take it all into a syringe and filter it through a 5 µm filter and a 0.2 µm filter into a sample tube.  
Syringe, 5 µm and a 0.2 µm filter will be cleaned with deionized water after each sample.

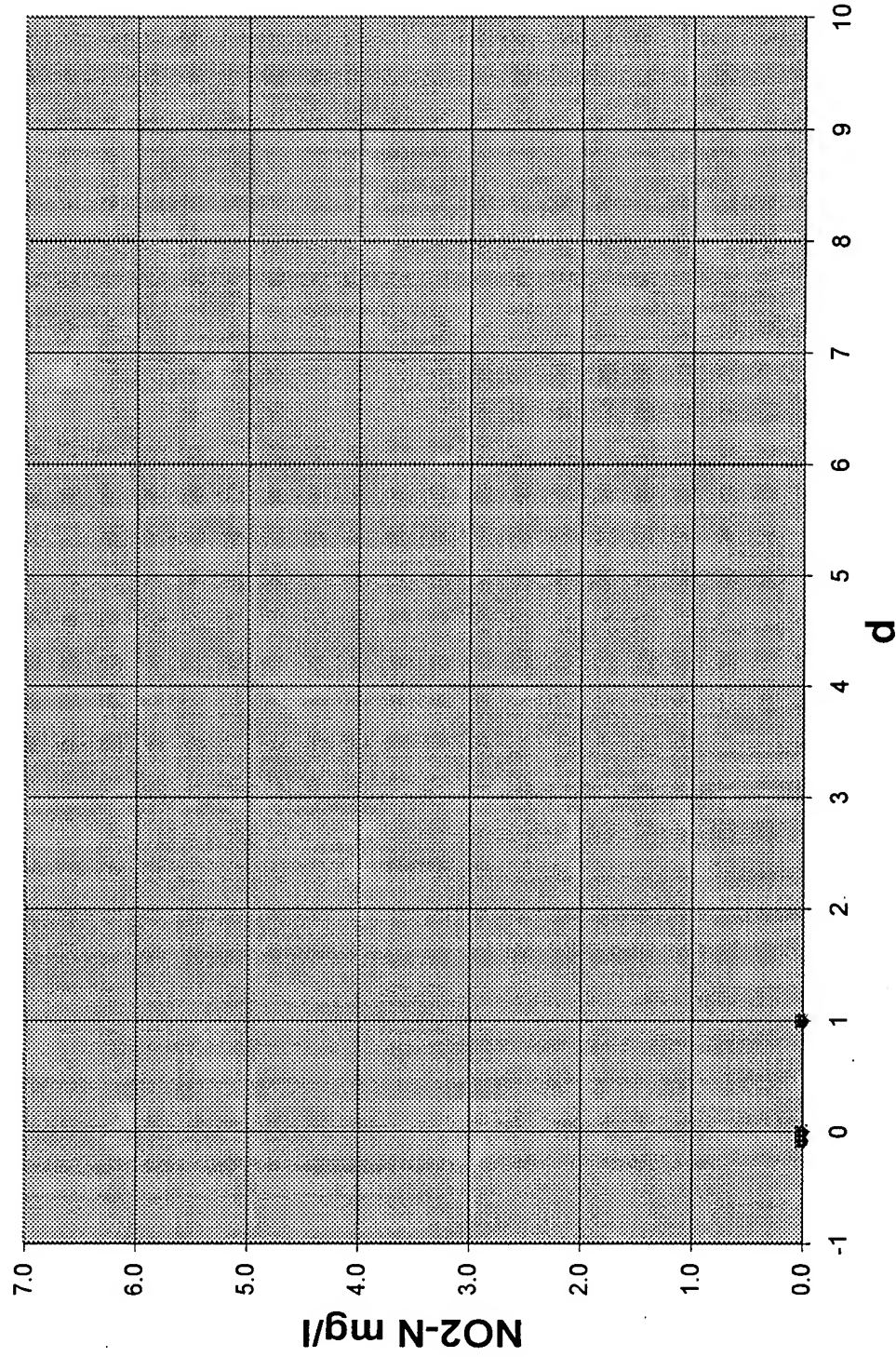
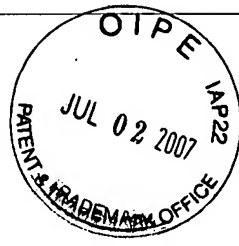
### Acceptance Criteria:

Ammonia-N should reach 0mg/L by day 5.

Nitrite-N should reach 0mg/L by day 8.

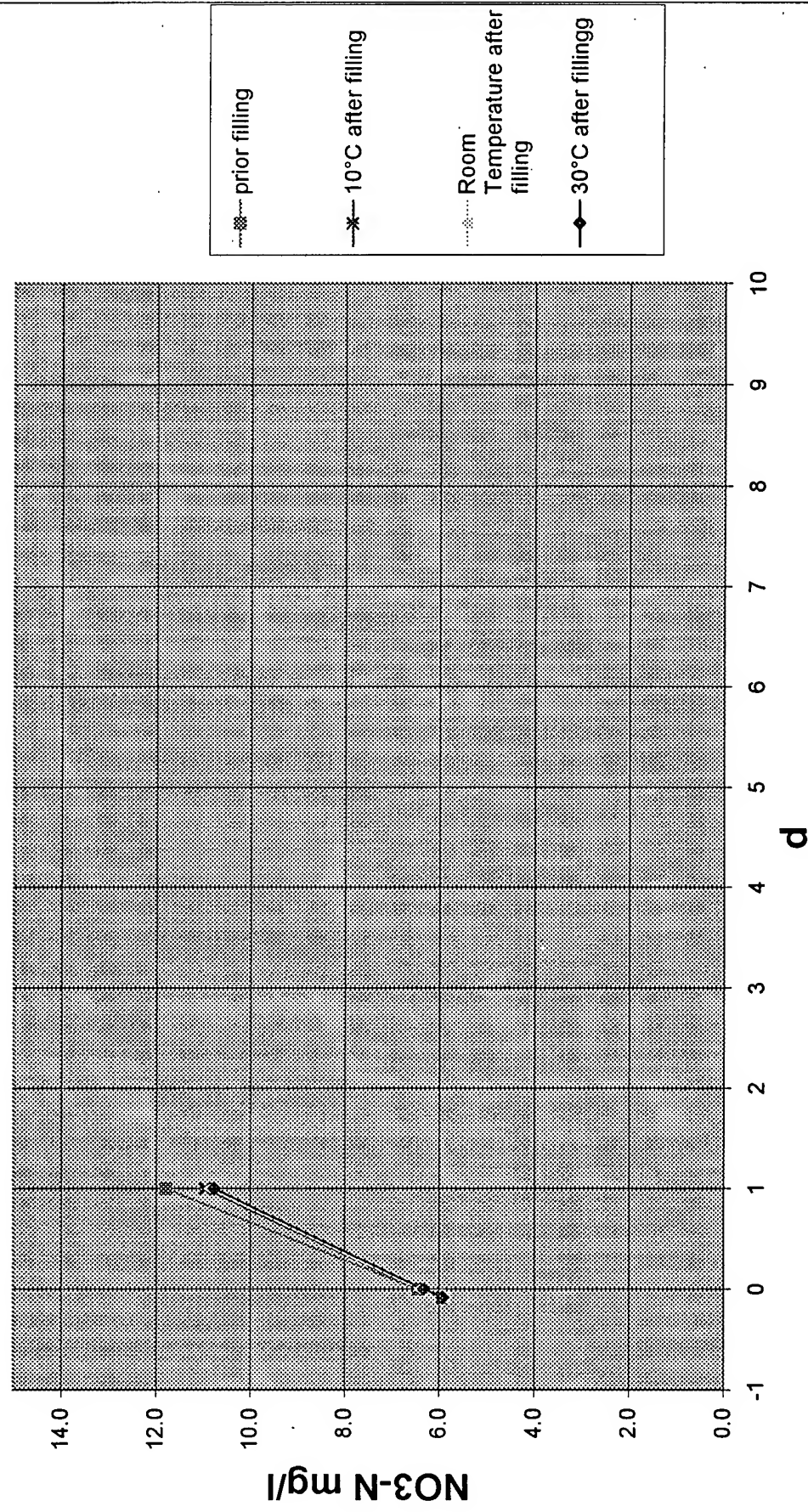
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## Nitrite Formation over Time



## Erlenmeyertest 10, Charge 148563

### Nitrate Formation





## Erlenmeyertest 10, Charge 148563

### Ammoniumabbau

